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TITLE: Understanding the role of TSC1/2 in cerebellar Purkinje neurons

PRINCIPAL INVESTIGATOR: Mustafa Sahin

CONTRACTING ORGANIZATION: Childrens Hospital Corporation

Boston, MA 02115

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PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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6. AUTHOR(S)

Mustafa Sahin

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

CHILDREN'S HOSPITAL CORPORATION,
THE OFFICE OF SPONSORED PROGRAMS
300 LONGWOOD AVE, BOSTON MA 02115-5724

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Tuberous sclerosis complex is a multisystem autosomal dominant disorder caused by mutations in either TSC1 or TSC2 genes. Previously our group has shown that Tsc1 knock-out mice Purkinje cells are involved in the development of autistic-like features for these mice. In addition, at BCH we have collected fibroblasts and derived pluripotent stem cell lines from TSC-patients and unaffected familial controls. We have developed differentiation protocol for generation of human Purkinje cells from iPSCs. We have studied mTOR-pathway hyperactivation in TSC2-deficient patient iPSC-derived PCs compared to control cells. We are also analyzing the proliferation and differentiation rate of TSC2-deficient neural precursor cells compared to control cells and studying electrophysiological properties of TSC2-deficient iPSC derived Purkinje cells. According to our preliminary funtional data, the TSC-patient iPSC-derived PCs and healthy control iPSC-derived PCs exhibit GABAergic inhibitory synaptic currents, which can be blocked with bicuculline. These cells also exhibit glutamatergic excitatory synaptic currents, which can be blocked with CNQX. The disease phenotyping with TSC deficient iPSC-derived PCs is important for the future development of new pharmacotherapy for TSC-patients with autism.

15. SUBJECT TERMS

autism, tuberous sclerosis, cerebellum

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified		19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Tuberous sclerosis complex (TSC) is a multisystem autosomal dominant disorder caused by mutations in either *TSC1* or *TSC2* genes. TSC patients are often affected with developmental delay and epilepsy and approximately half of patients with TSC display symptoms of autism spectrum disorder (ASD). Although much research has been conducted, the neural circuitry and molecular mechanism underlying autism remain unclear. Specific cerebellar defects have been seen in TSC patients, suggesting a crucial role for the cerebellum. Cerebellar pathology can be found in 1 in 3 patients with TSC, and studies correlate cerebellar pathology with ASD symptomatology in patients with TSC. Purkinje cells are the sole output neuron of the cerebellum, and previously we have shown that *Tsc1* mutant Purkinje cells cause autistic-like behaviors in mice. The objective of this study is to establish a novel platform to characterize *TSC1/2*-mutation related neurodevelopmental disorders and ASD related cellular dysfunctions in *in vitro* and *in vivo* models of human patient specific iPSC-derived Purkinje cells. The results of this study will provide important insights about the molecular mechanism underlying neurodevelopmental disorders. In the future, the results of this study can be used to establish novel therapeutic targets for treatment of TSC patients diagnosed with or at risk of developing ASD.

2. KEYWORDS:

Human iPSC, TSC1/2, Purkinje cells, disease phenotyping, autism, cerebellum.

3. ACCOMPLISHMENTS:

Specific Aims:

Aim 1: To compare the transcriptional profiles of mouse and human PCs with and without TSC1/2 expression. As a first step, we will carry out RNAseq in PC's isolated from Tsc1-knockout mouse and control mice and compare the gene expression profiles to identify differences in expression of transcription factors during PC development. Using the protocol developed by the Hatten lab for differentiating hES cells into PCs, we will select iPSC clones that express the Pcp2 bacTRAP tag (*Pcp2-Egfp-L10a*), differentiate them into PCs and assay the transcriptomes of iPSC-derived PCs from TSC and unaffected patients. We will then use bioinformatics to identify gene pathways that differ in Tsc1 mutant mouse and human PCs compared with wild type/unaffected cells.

Aim 2: To genetically correct TSC mutations in patient-specific iPSC lines and rescue the disease phenotypes in patient specific neurons *in vitro*. We will employ CRISPR-Caspase 9 (Cas9) genome editing techniques to correct TSC1/2 mutations in patient-specific iPSC lines and evaluate the reversibility of any observed phenotypes. The TSC1/TSC2-deficient iPSCs will be differentiated into neuronal cells to establish comprehensive cellular phenotypic analysis compared to isogenic controls, including cell vulnerability to a range of insults, oxidative stress, and electrophysiological properties reflecting cellular excitability.

Aim 3: To evaluate functionality and neural circuit formation capacity of human patient derived cerebellar precursor and PCs *in vivo* in Tsc1-PC knockout mice. The goal is to characterize the functionality and differentiation capacity of human iPSC-derived cerebellar precursors and PCs *in vivo*. We will study if human TSC patient neurons and control neurons survive, and develop normally, integrate into the correct layer of the mouse cerebellum and show functionality. We will analyze dendritic arborization, axon outgrowth, and use

electrophysiology to measure neurons excitability. If successful, this research will provide a critical new model system for analyzing disorders that involve human cerebellar circuit development. This study will provide proof of concept that loss of PCs is responsible for the behavioral deficits in the Tsc-deficient animals.

Studies and Results

For Aim 1. We have sorted GFP+ L7 Cre+ Tsc1-mutant and control mice Purkinje cells and performed transcriptional profiling with Illumina RNA sequencing. We have identified 64 significantly downregulated genes in Tsc1-mutant Purkinje cells compared to controls and 18 significantly upregulated genes in Tsc1 mutants compared to controls. The genes identified with this analyses strongly correlate with the electrophysiological deficits previously reported with Tsc1-mutant mice PCs. In addition, we have identified with QRT-PCR that some of these genes are also expressed in TSC2-deficient patient iPSC-derived PCs. Pcp2-EGFP-L10a TRAP hPSC lines for transcriptome analysis of differentiated PCs are being generated at Rockefeller.

For Aim 2. We have collected fibroblasts and derived pluripotent stem cell lines from TSC-patients and unaffected familial controls. These patients have been clinically diagnosed with TSC, have ASD diagnosis, and carry known *TSC2* mutations that have been sequenced. We have developed new differentiation protocol for generation of Purkinje cells from the iPSCs with cell sorting and immunopanning method for Thy1+ cells. According to our preliminary results the differentiation of human Purkinje cells requires long-differentiation time and co-culturing with mouse granular neurons, the whole differentiation protocol lasts up to 140 days *in vitro.* Work at Rockefeller is continuing to explore ways to speed up maturation of hPSC-PCs, with stereotypical PC morphology seen as early as 25 days after isolation and co-culture with mouse granule cells (Figure 1).

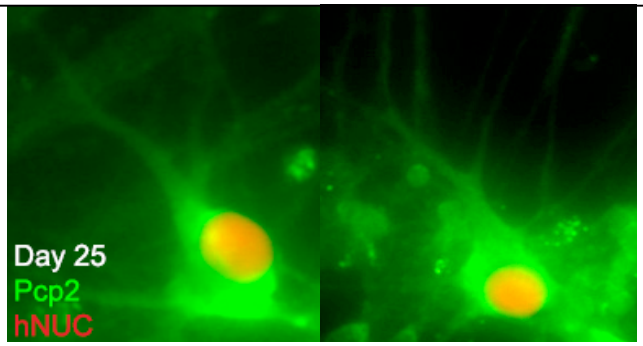

Figure 1. hESC-derived PCs after Thy1+ selection in co-cultures with mice cells.

So far, we have detected the mTOR-pathway hyperactivation in TSC2-deficient patient iPSC-derived PCs with increased levels of phosphorylated ribosomal protein S6 and phosphorylated p70-S6 kinase (Figure 2). According to our data, the mTOR-pathway hyperactivation increased the neural precursor cell proliferation capacity in TSC2-deficient cell population compared to control cells. We have tested pharmacological rescue of the mTOR-pathway hyperactivation by treating the TSC-patient iPSC-derived PCs with rapamycin (20nM) for two weeks. Rapamycin treatment decreased the phospho-S6 levels in three different TSC-patient lines (SAH0047-01, CRA401 and 77) to similar levels as seen with control-iPSC-derived PCs (SAH0047-02 control, Male654 control, 78 control) and also reduced the proliferation rate of these cells.

Oour preliminary data shows that iPSC derived PCs are spontaneously active electrophysiologically. We are in process of putting together a manuscript describing the

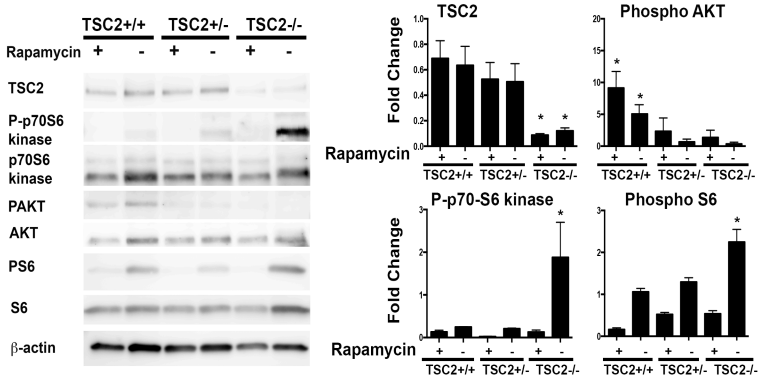

Figure 2. TSC2 deficient patient cells have decreased TSC2 expression and increased phosphorylation of p70-S6-kinase and ribosomal protein S6. Phosphorylation of AKT is decreased in TSC2-deficient cells compared to control cells. *p<0.05.

phenotypic characterization of TSC-patient derived Purkinje cells *in vitro*. In the future, we are planning to extend our experiments with additional TSC-patient iPSC-lines and TSC2-null iPSC-lines. We are also going to focus on detailed characterization of the molecular mechanism underlying the disease phenotype *in vitro* with transcriptional profiling.

For Aim 3. We have begun developing methods for implanting hPSC-PCs into the neonatal mouse cerebellum. Preliminary results show good survival and proper targeting of hPSC-PCs into the cerebellum four days post implantation (Figure 3).

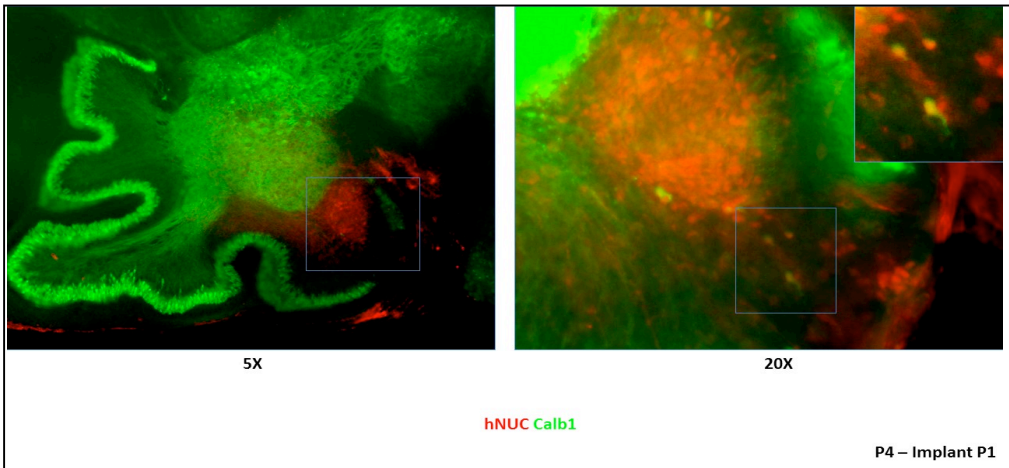

Figure 3. hPSC-PCs implantation into the neonatal mouse cerebellum. Human cells are marked by human nuclear antigen (hNUC) and native mouse PCs are marked by Calb1. Some human cells are beginning to express Calb1 (inset, right).

What opportunities for training and professional development has the project provided?

During this period Dr. Sundberg has participated in the Clinical Translation Workshop provided by ISSCR, 06/23/2015. This course covered human stem cell derived neural cell production for disease phenotyping and drug screening development.

How were the results disseminated to communities of interest?
Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?
For Aim 1. We plan to use transcriptional profiling in order to characterize the TSC2-deficient patient iPSC-derived PCs co-cultured with mouse granular neurons.

For Aim 2. We are going to utilize CRISPR/Cas9 technology to genetically correct the TSC-patient iPSC lines in order to create isogenic controls and we are also going to produce double TSC2 knock-out iPSC-lines for our future studies.

For Aim 3. We are planning to start optimizing transplantation of TSC-patient iPSC-derived PCs to Tsc1-KO mice cerebellum.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?
Nothing to Report.

What was the impact on other disciplines?
Nothing to Report.

What was the impact on technology transfer?
Nothing to Report.

What was the impact on society beyond science and technology?
Nothing to Report.

5. CHANGES/PROBLEMS: The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

- Changes in approach and reasons for change.
- Actual or anticipated problems or delays and actions or plans to resolve them.
- Changes that have a significant impact on expenditures.
- Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents.

Changes in approach and reasons for change
Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them
Nothing to report.

Changes that had a significant impact on expenditures
Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report.

6. PRODUCTS

Publications, conference papers, and presentations
Nothing to report

Journal publications.
Nothing to report

Books or other non-periodical, one-time publications. Nothing to report.

Other publications, conference papers, and presentations. Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.

- **Website(s) or other Internet site(s)**
  Nothing to report.

- **Technologies or techniques**
  Nothing to report.

- **Inventions, patent applications, and/or licenses**
  Nothing to report.

- **Other Products**
  Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS
Provide the following information on participants:

- what individuals have worked on the project?
- has there been a change in the other active support of the PD/PI(s) or senior/key personnel since the last reporting period?
- what other organizations have been involved as partners?

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort).
• Provide the name and identify the role the person played in the project. Indicate the nearest whole person month (Calendar, Academic, Summer) that the individual worked on the project. Show the most senior role in which the person worked on the project for any significant length of time. For example, if an undergraduate student graduated, entered graduate school, and continued to work on the project, show that person as a graduate student, preferably explaining the change in involvement.
Describe how this person contributed to the project and with what funding support. If information is unchanged from a previous submission, provide the name only and indicate “no change”.

This is a collaborative project between Boston Children’s Hospital and Rockefeller University.

Name: Mustafa Sahin
Project Role: PI
Nearest person month worked: 1.2 cal
Contribution: As PI, Dr. Sahin has supervised all aspects of the research plan and coordinated the communication with Dr. Hatten’s lab at Rockefeller University.

Name: Maria Sundberg
Project Role: postdoctoral fellow
Nearest person month worked: 6 cal
Contribution: no change

Name: Mary Elizabeth Hatten
Project Role: Co-PI
Nearest person month worked: 1.2 cal
Contribution: Dr. Hatten supervised the work done on Aims 1,2 and 3 and coordinated the research plan with Dr. Sahin at Boston. She also traveled to Boston to meet with personnel there to review progress as well as to plan a publication that is currently in preparation.

Name: David Buchholz
Project Role: Subcontract Postdoctoral Fellow
Nearest person months worked: 6 cal
Contribution: Dr. Buchholz carried our the progress reported in Aims 1,2 and 3 on generating hES cell lines expressing a Pcp2 TRAP line, on further developing a protocol for differentiating Purkinje neurons and on developing a methodology to implant immature hES-derived PCs into mouse cerebellar cortex.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

If there is nothing significant to report during this reporting period, state "Nothing to Report."
If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Name: Mustafa Sahin
Project Role: PI

New Active Support:

Human Neuron Core at Boston Children's Hospital (PI: Sahin)
Massachusetts Life Science Center
1000 Winter Street, Suite 2900
Waltham, MA 02451
Contracting/Grant Officer: Brad Rosenblum
Email: brosenblum@masslifesciences.com
07/01/2015 – 06/30/2018 – 0.60 calendar months
$431,500/year
The overall goal for this grant is to build a state of the art human neuron core lab at Boston Children's Hospital.
No overlap.

Sonic Hedgehog and Ciliary Signaling in TSC (PI: Sahin)
Tuberous Sclerosis Alliance (TSA)
801 Roeder Road, Suite 750
Silverspring, MD 20910; Phone: 301-562-9890.
Contracting/Grant Officer: Kari Luther Rosbeck (CEO)
12/01/2015 – 11/30/2017 – 0 calendar months
$75,000/year
This proposal aims to determine the involvement of Shh signaling in altered neuronal maturation and ciliation of TSC and to dissect the translational relevance of altered Shh and ciliary signaling in the CNS pathology of TSC.
No overlap.

A Phase 2b placebo-controlled cross-over study of the rh-IGF1 (mecasermin [DNA] injection) for treatment of Rett syndrome and development of Rett-specific novel biomarkers of cortical and autonomic function (PI: Sahin)

International Rett Syndrome Foundation
4600 Devitt Drive
Cincinnati, OH 45246
Contracting/Grant Officer: Janice Ascano, PhD
Email: jascano@rettsyndrome.org Phone: 1-615-283-5943
08/01/2012 – 12/31/2016 – 0.36 calendar months
$193,524/year
This source is providing the main financial support for a randomized, double-blind, placebo-controlled, two-arm crossover study of IGF-1 in 30 children with Rett Syndrome. We will collect extensive data on both efficacy and safety. The primary outcome measure is improvement in autonomic and respiratory function.
No overlap.

Natural History of Rett Syndrome, MECP2 Duplications, and Rett-Related Disorders (Co-PI: Sahin)
NIH U54HD061222-12
National Institutes of Neurological Disorders and Stroke
6001 Executive Boulevard, Suite 3290, MSC 9537
Bethesda, MD 20892-9537 Contracting/Grants Officer: Vicky R Haines
Email: vhaines@mail.nih.gov Phone: 301-496-1365
09/30/2003-07/31/2019 - 0.12 calendar months
Rare Disease multisite initiative to provide natural history studies leading to possible therapies for Rett Syndrome, MECP2 Duplication Disorder, and Rett-related Disorders Natural History.
No overlap.

Molecular Profiling of the Tuberous Sclerosis Brain and Patient Blood Cells (PI: Sahin)
F. Hoffmann-LaRoche, LTD
Grenzacherstrasse 124
4070 Basel, Switzerland
Contracting/Grants Officer: Jason Hannon
03/01/2016 – 03/18/2018 – 0.12 calendar months
$127,717/year
The overall goal of this study is to generate a comprehensive molecular profile of the cortical-inhabitory and excitatory neurons and cerebellar Purkinje cells from rodent wild type and TSC modulated brain and to perform molecular profiling of blood cells from TSC patients.
No overlap.

A Randomized, Double-Blind, Placebo-Controlled, Dose-Ranging Study of the Safety and Pharmacokinetics of Oral NNZ-2566 in Pediatric Rett Syndrome (Co-PI: Sahin)
Neuren Pharmaceuticals, LTD
Suite 501, 697 Burke Road
Camberwell, VIC 3124, Australia
Contracting/Grants Officer: Nancy Jones, PhD
Email: njones@neurenpharma.comPhone: +44 (0) 121 449 7381
03/01/2016 – 02/28/2017 – 0.12 calendar months
$111,013/year
A randomized, double-blind, placebo-controlled dose ranging study of the safety and tolerability of NNZ-2566, also known as trofinetide, in female children and adolescents with Rett syndrome. This study will also investigate measures of efficacy and the pharmacokinetics of NNZ-2566 during treatment.
No overlap.

Preventing Epilepsy Using Vigabatrin in Infants with Tuberous Sclerosis Complex (PREVeNT Trial) (Co-PI: Sahin)
NIH 1U01NS092595-01A1
National Institutes of Neurological Disorders and Stroke
6001 Executive Boulevard, Suite 3290, MSC 9537
Bethesda, MD 20892-9537 Contracting/Grants Officer: Vicky R Haines
Email: vhaines@mail.nih.gov Phone: 301-496-1365
07/01/2015 – 06/31/2020 – 0.60 calendar months
$78,710/year

Dr. Sahin will be responsible for the recruitment and enrollment of subjects at BCH for participation in this trial. **No overlap.**

New Completed projects:

Intellectual and Developmental Disabilities Research Center, (PI: Scott Pomeroy; Component Director, Translational Neuroscience Sub core: Sahin)
NIH/NICHD P30 HD018655-32
Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) 31 Center Drive, Building 31, Room 2A32 Bethesda, MD 20892-2425
Contracting/Grant Officer: Hong Cao
Email: hongca@mail.nih.gov Phone: 301-435-6999
08/01/1997 - 06/30/2016 – 0.24 calendar months
$992,309 DC - Entire Grant
This grant provides state-of-the-art translation core facilities to all projects within the IDDRC program.

Potential EEG Biomarkers and Antiepileptogenic treatment strategies-TSC, (Co-Investigator: Sahin)
NIH 1P20NS080199-01 (Sub from UAB – PI M. Bebin)
The Board of Trustees of the UAB 15303 3rd Avenue South, AB 1170
Birmingham, AL 35294-0111
Contracting/Grant Officer: Lynn W. Stedman, OSP Phone: 205-934-5266 Email: osp@uab.edu
09/01/2012-08/31/2016 (NCE ended) – 0.6 calendar months
$35,655/year
Specific Aims:
Aim 1. (Clinical Core): To determine whether EEGs during infancy is a reliable biomarker to identify TSC patients that will develop infantile spasms/epilepsy in the near future and thus are appropriate candidates for an antiepileptogenic drug trial. Aim 2. (Preclinical Core): To determine the optimal rapamycin reatment paradigms that maintain antiepileptogenic efficacy but minimize risks of side effects. Aim 3. Administrative Core): To establish a network of research sites that can most effectively and efficiently facilitate both pre-clinical and clinical studies for antiepileptogenic drug trials in TSC.

Role of CTGF in White Matter Development in Tuberous Sclerosis, (PI: Sahin)
DOD X81XWH-13-1-0040
Department of Defense
USA Med Research ACQ Activity
820 Chandler St. Fort Detrick MD 21702-5014
Contracting/Grant Officer: Dawn Hurley/ Catherine G. Baker Email: Catherinegbaker@amedd.army.mil
02/01/2013- 01/31/2016 – 0.96 calendar months
$141,667/ Year
The overall goal of this grant is to better characterize the role of CTGF in TSC-related white matter deficits and to explore its potential as a therapeutic target. We propose two independent but complementary sets of experiments. Specific Aims: Aim 1. To determine the role of CTGF in hypomyelination (A) in the TSC mouse model and (B) in human TSC brain. Aim 2. To examine the mechanisms by which CTGF regulates oligodendrocyte differentiation.

Cpg15/Neuritin as a potential therapeutic target in Spinal Muscular Atrophy (SMA), (PI: Sahin)
Shire
Shire Human Genetic Therapies,
125 Spring Street; Lexington MA, 02421
Contracting/Grants Officer: Albert Seymour (VP- Drug Discovery and Translational Research)
09/01/2013- 08/31/2015 – 1.2 calendar months
$122,319 /Year
The goal of this project is to develop new therapeutics for Spinal Muscular Atrophy.

Mechanical Characterization of Brain Tissue and Individual Neurons in Autism Models

Simons Foundation
160 Fifth Avenue, 7th Floor, New York, NY 10010
Contracting/Grants Officer: Calissia R. Franklyn, Grants Associate,
Phone: 212-604-8056, Email: calvarezfranklyn@simonsfoundation.org
06/01/2014- 05/31/2015 – 0.12 calendar months
$34,918/year
Specific Aims:
Aim 1. Characterize nano-to micro-scale mechanical properties of brain tissue from the rodent models of ASD, and correlate these with myelination extent and neuronal organization. Aim 2. Determine the effect of extracellular material stiffness on connectivity and function of neurons. Aim 3. Characterize physical and mechanical properties of individual neurons from the autistic rodents.

TSC Autism Center of Excellence Research Network (TACERN) Research Consortium, (PI: Sahin)
TS Alliance
801 Roeder Road, Suite 750
Silver Spring, MD 20910
Contracting/Grants Officer: Kair Luther Rosbeck (CEO)
01/01/2012 – 06/30/2015. NCE – 0.06 calendar months
$64,715/year
The goal of this project is to accelerate the start-up and coordination of two studies planned by the TSC Autism Center of Excellence Research Network (TACERN) Research Consortium.

Name: Mary E. Hatten
Project Role: Co-PI

New Active Support:

Starr Tri-Institutional Stem Cell Initiative Hatten (PI) 07/01/2016- 6/30/2019
Funding Agency: Starr Foundation
Title: Role of Tet and Chromatin Remodeling Genes in Human Cerebellar Neuron Synapse Formation and Function.
The major goal of this project is to study the effects of genetic perturbation of chromatin-modifying factors on the differentiation and synaptic physiology of hESC-derived cerebellar neurons. We will use CRISPR/Cas9 constructs to remove the Tet and other chromatin remodeling genes in hESC-derived GCs and PCs. Subsequently we will express these constructs in hESC-GCs or PCs and assay whether targeting Tet or chromatin remodeling genes affects cerebellar synapse formation when the human cells are co-cultured with mouse target For targeted genes that decrease synapse formation by 50%, Hatten will use TRAP methodology to assay changes in gene expression, focusing on changes in axon guidance (dendrite formation) and ion channel genes, genes that we previously showed are altered by activation of Tet1/3 (Xhu et al, 2016). We will also transplant relevant differentiated hESC clones into mouse cerebellum to test their ability to integrate and form dendrites. Finally, Ryan will use a suite of biophysical approaches to examine the functional properties of synapses formed between hESC-derived cerebellar neurons and defined postsynaptic targets in vitro.
Role: PI (Co-PIs, Joseph G. Gleeson, M.D.; Timothy A. Ryan, Ph.D.)

Completed Support:
A Cerebellar Mutant for Investigating Mechanisms of Autism in Tuberous Sclerosis, (PI: Sahin)
Autism Speaks
1060 State Road, 2nd Floor Princeton, NJ 08540 Contracting/Grant Officer: Joan New
Phone# 609-228-7313 Email: Jnew@autismspeaks.org 02/01/2012- 01/31/2015 – 1.2 calendar
$136,325/year Specific Aims:
1. Evaluate whether abnormal neuronal connectivity contributes to Purkinje Cell Tsc1 mutant phenotype
2. Evaluate critical periods in ASDs in Purkinje Cell Tsc1 mutants.

Role of CTGF in White Matter Development in Tuberous Sclerosis, (PI: Sahin)
DOD X81XWH-13-1-0040
Department of Defense

USA Med Research ACQ Activity
820 Chandler St. Fort Detrick MD 21702-5014 Contracting/Grant Officer: Dawn Hurley/ Catherine G. Baker
Email: Catherinegbaker@amedd.army.mil
02/01/2013- 01/31/2016 – 0.96 calendar
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The overall goal of this grant is to better characterize the role of CTGF in TSC-related white matter deficits and to explore its potential as a therapeutic target. We propose two independent but complementary sets of experiments.
Specific Aims:
Aim 1. To determine the role of CTGF in hypomyelination (A) in the TSC mouse model and
(B) in human TSC brain.
Aim 2.To examine the mechanisms by which CTGF regulates oligodendrocyte differentiation.

Cpg15/Neuritin as a potential therapeutic target in Spinal Muscular Atrophy (SMA), (PI: Sahin)
Shire
Shire Human Genetic Therapies,
125 Spring Street; Lexington MA , 02421
Contracting/Grants Officer: Albert Seymour (VP- Drug Discovery and Translational Research) 09/01/2013-08/31/2015 – 1.2 calendar
$122,319 /Year
The goal of this project is to develop new therapeutics for Spinal Muscular Atrophy.

5R01NS051778-09 Hatten (PI) 05/01/2011-02/29/2016
Funding Agency: NIH/NINDS

Title: Role of Cdc42 and Par6 Polarity Complex in CNS Neuronal Migration
The goal of this research is to examine the role of the mPar6α polarity complex in the migration of CNS neurons along glial fibers during cerebellar development. The specific aims of this research are to investigate the other components of the mPar6α complex, the atypical PKCζ and Par3, to use genetic methods for chromophore-assisted inactivation of mPar6α in granule neurons and to examine upstream and downstream signaling pathways for the mPar6α complex in CNS glial-guided migration.
Role: PI

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."
Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Rockefeller University (as above, no change)

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- Financial support;
- In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);
- Facilities (e.g., project staff use the partner's facilities for project activities);
- Collaboration (e.g., partner's staff work with project staff on the project);
- Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and
- Other.

8. SPECIAL REPORTING REQUIREMENTS: None

9. APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text.
Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

None